

Activation of protein C and hemodynamic recovery after coronary artery bypass surgery

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Objectives: Activated protein C is a physiologic anticoagulant that is activated by thrombin and upregulated during coronary artery bypass grafting. We studied the balance between thrombin generation and activated protein C levels during coronary artery bypass grafting and hypothesized that protein C activation during reperfusion is associated with hemodynamic recovery or postoperative myocardial damage.

Methods: One hundred patients undergoing elective on-pump coronary artery bypass grafting were prospectively studied. Activated protein C, protein C, prothrombin fragment F1+2 (a marker of thrombin generation), and D-dimer (a marker of fibrinolysis) levels were measured preoperatively and at 7 time points during cardiopulmonary bypass and reperfusion and postoperatively. Hemodynamic parameters were measured serially. Cardiac biomarkers (mass of the Mb fraction of creatine kinase and troponin T) were measured postoperatively.

Results: Reperfusion induced a significant increase in thrombin generation. Activated protein C levels peaked after heparin neutralization, when they increased more than 3-fold. Activated protein C levels correlated with F1+2 and D-dimer levels during cardiopulmonary bypass and reperfusion. Even though this correlation peaked during early reperfusion ($r = 0.55$, $P < .001$), the ratio of activated protein C to F1+2 decreased during surgical intervention and early reperfusion by 70% from the preoperative level, indicating a marked delay in protein C activation in relation to thrombin generation. Patients in the highest quintile of activated protein C levels during this period had a higher postoperative cardiac index (mean, $3.1 \text{ vs } 2.5 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$; $P < .05$) and lower systemic vascular resistance (mean, $2137 \text{ vs } 2429 \text{ dyne} \cdot \text{s} \cdot \text{cm}^{-5} \cdot \text{m}^{-2}$; $P < .05$). Conversely, levels of preoperative activated protein C and activated protein C measured after heparin neutralization were associated with unfavorable hemodynamic recovery postoperatively. Activated protein C or protein C levels were not associated with increased postoperative cardiac biomarkers.

Conclusions: Reperfusion caused significant thrombin generation that was followed by activation of protein C. The balance of activated protein C with thrombin is associated dynamically with postoperative hemodynamic recovery.

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Activated protein C (APC) is a physiologic anticoagulant that also possesses anti-inflammatory and antiapoptotic properties.^{1,2} Protein C is activated by thrombomodulin-bound thrombin. Initial generation of thrombin activates protein C, which further regulates thrombin formation. Initial thrombin generation can therefore have a net antithrombotic effect. Propagated thrombin levels, on the other hand, are procoagulant, antifibrinolytic, and proinflammatory, despite the activation of anticoagulant pathways.³ The dynamic balance between thrombin generation and protein C activation is of major importance in many clinical situations.

Abbreviations and Acronyms

AMI	= acute myocardial infarction
ANOVA	= analysis of variance
APC	= activated protein C
CABG	= coronary artery bypass grafting
CI	= cardiac index
CK-Mbm	= mass of the Mb fraction of creatine kinase
CPB	= cardiopulmonary bypass
ICU	= intensive care unit
PVRI	= pulmonary vascular resistance index
SD	= standard deviation
SVI	= stroke volume index
SVRI	= systemic vascular resistance index
TnT	= troponin T

After experimental work that showed that APC prevented mortality in a baboon model of bacteremia,⁴ APC has emerged as a therapeutic option in human subjects. In adult patients with sepsis, recombinant human APC reduced mortality and the incidence of organ failure.^{5,6} Experimental studies have shown evidence for beneficial effects of APC in various models of ischemia-reperfusion.⁷ However, the complicated endothelial process of protein C activation, which results in on-demand negative feedback in response to thrombin generation, might be disturbed by simultaneous activation of inflammation, as in sepsis⁸ or severe pancreatitis.⁹

Cardiopulmonary bypass (CPB) and coronary artery bypass grafting (CABG) cause significant activation of both coagulation and inflammation. During CPB, high levels of both thrombin and APC are generated, and reperfusion after CABG further causes a rapid and distinct increase in both thrombin and APC levels.^{10,11} Protein C activation after CABG had an inverse correlation with neutrophil sequestration in the human myocardium and with L-selectin expression of circulating neutrophils, implicating APC as a possible regulator of myocardial ischemia-reperfusion injury.¹² In a previous study we showed that thrombin generation during reperfusion after CABG is associated with postoperative myocardial damage and increased pulmonary vascular resistance.¹⁰ The same patient population was prospectively studied in this study. Based on our finding that thrombin is associated with myocardial damage and a previous smaller study in which APC was associated with favorable hemodynamic recovery after CABG,¹¹ our aim in this study was to investigate the associations of protein C activation with postoperative hemodynamic recovery and myocardial damage.

Methods**Study Setting and Patients**

This was a prospective, single-center study of 100 consecutive patients who were scheduled for primary, elective, on-pump CABG. Exclusion criteria were as follows: use of warfarin, unfractionated or low-molecular-weight heparin, or aspirin less than 5 days before surgical intervention; renal failure; abnormal preoperative international normalized ratio; anemia; and thrombocytopenia. Institutional ethical committee approval and written informed consent from each patient were obtained. Anesthesia, CPB, transfusions, hemodynamic management, fluid therapy, and postoperative medication followed a prospective clinical protocol, as previously described.¹⁰ The demographic and operative data have been previously reported.¹⁰ Operative risk was evaluated according to the EuroSCORE,¹³ which takes into account several patient-related, cardiac, and operation-related preoperative risk factors, including age; sex; the presence of pulmonary, neurologic, and renal comorbidity; the presence of extracardiac arterial disease, previous cardiac surgery, active endocarditis, unstable angina, left ventricular dysfunction, recent myocardial infarction, and pulmonary hypertension; urgency; and the type of operation performed.

CPB and Surgical Intervention

Details have been reported previously.¹⁰ During CPB nonpulsatile pump flow, noncoated circuits and a membrane oxygenator (Trilium Affinity; Medtronic, Minneapolis, Minn) were used in conjunction with moderate systemic hypothermia. Pump flow was maintained at $2.4 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$. Five thousand international units of unfractionated heparin was added to the priming solution, and an initial intravenous dose of 400 IU/kg heparin was administered. Heparinization was monitored with kaolin-activated clotting time measurements every 20 minutes. Activated clotting time was maintained at greater than 600 seconds by means of additional doses of 5000 IU of heparin. During CPB, blood suctioned from the operative field was returned to the systemic circulation through a filtered cardiectomy reservoir throughout the operation until protamine administration. After CPB, the content of the extracorporeal circuit was collected into nonanticoagulated blood bags and returned to the patient. Heparinization was neutralized with 1 mg of protamine sulfate per 100 IU of heparin.

After median sternotomy, the left internal thoracic artery, right internal thoracic artery, saphenous vein, or radial artery grafts were harvested. The ascending aorta and the right atrial appendage were cannulated, and CPB was initiated. Intermittent antegrade cold ($+10^{\circ}\text{C}$ - 12°C) blood cardioplegia was used for myocardial protection. The aorta was crossclamped during suturing of all anastomoses. The left internal thoracic artery was anastomosed to the left anterior descending coronary artery in all cases except one. Four patients also received a right internal thoracic artery graft. Additional aortocoronary anastomoses were performed by using saphenous vein or radial artery grafts. Intraoperative volume-flow measurements of all grafts were performed with a transit-time flowmeter (Medi-Stim Butterfly Flowmeter; Medi-Stim AS, Oslo, Norway) to ascertain immediate graft patency.

Hemodynamic Measurements

A radial artery cannula and a pulmonary artery catheter were inserted, and heart rate, arterial pressure, central venous pressure,

pulmonary artery pressure, and pulmonary capillary wedge pressure were measured. Cardiac output measurements were performed with thermodilution. Mean arterial pressure, mean pulmonary artery pressure, stroke volume, stroke volume index (SVI), cardiac index (CI), systemic vascular resistance index (SVRI), and pulmonary vascular resistance index (PVRI) were calculated with standard formulas at 5 time points: preoperatively (coinciding with time point A for blood sampling, see below), after termination of CPB (time point E), on arrival in the intensive care unit (ICU), 6 hours after protamine (time point F), and on the first postoperative day (time point G).

Hemodynamic Management

When the postoperative CI value was less than $2.0 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$, pulmonary capillary wedge pressure was first adjusted to 12 to 15 mm Hg by optimizing preload with an infusion of Ringer's acetate, 6% hydroxyethyl starch, or 4% human albumin. If the CI value remained less than $2.0 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$, epinephrine ($0.02\text{--}0.2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was infused. If the CI value still remained less than $2.0 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$, a milrinone infusion ($0.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was added. When the mean arterial pressure was less than 70 mm Hg, preload was first optimized (see above), and when necessary, norepinephrine ($0.01\text{--}0.1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was infused.

Preoperatively, after the induction of anesthesia (corresponding to time point A, see "Hemodynamic measurements" above and "Blood samples" below), 28% of the patients required a norepinephrine infusion, and none of the patients received either epinephrine or milrinone. During CPB, before the release of the aortic clamp (time points B and C), 77% of the patients received norepinephrine, 5% received epinephrine, and none of the patients received milrinone. After the release of the aortic clamp, during early reperfusion and 30 minutes after heparin neutralization (time points D and E), 90% of the patients received norepinephrine, 84% received epinephrine, and 7% received milrinone. Postoperatively, on arrival in the ICU, 75% of the patients received norepinephrine, 81% received epinephrine, and 7% received milrinone. Six hours after heparin neutralization (time point F), 87% of the patients received norepinephrine, 71% received epinephrine, and 6% received milrinone, and on the morning of the first postoperative day (time point G), 37% of the patients received norepinephrine, 27% received epinephrine, and 4% received milrinone.

Use of epinephrine during CPB (time points B and C) and during early reperfusion (time points D and E) was associated with higher postoperative PVRI and SVRI, respectively (repeated-measures analysis of variance [ANOVA]: $P = .003$ and $P = .014$, respectively). Use of norepinephrine preoperatively (time point A) and during CPB (time points B and C) was associated with higher postoperative SVI ($P = .042$ and $P = .046$, respectively). Use of milrinone during early reperfusion was associated with lower postoperative SVRI ($P = .036$). Otherwise, use of vasoactive medications was not associated with subsequent hemodynamic parameters.

Blood Samples

Blood samples were collected at 8 time points (points A-H): preoperatively (point A), at 15 minutes of CPB (point B), immediately before the release of the aortic clamp (point C), 15 minutes

after the release of the aortic clamp (point D), 30 minutes (point E) and 6 hours (point F) after protamine administration, and on the first (point G) and fifth (point H) postoperative days. Samples A through G were collected through a radial artery catheter, and sample H was collected either through an atraumatic venipuncture or through a radial artery catheter. Samples for measurement of F1+2 and D-dimer levels were collected into vacuum test tubes with 3.8% sodium citrate. For measurement of APC levels, a citrate benzamidine anticoagulant mixture was used according to the method of Gruber and Griffin.¹⁴ The first 5 mL of each sample were discarded. The samples were cooled on ice and centrifuged (1500g for 10 minutes) at $+4^{\circ}\text{C}$. Plasma was separated and stored at -80°C .

Laboratory Analyses

APC and protein C were analyzed with an enzyme capture assay, as previously described,¹⁴ and values are given as mean percentages of normal pooled human plasma. Prothrombin fragment F1+2 was analyzed with an enzyme-linked immunoassay (Enzygnost F1+2micro; Dade Behring, Marburg, Germany), as previously described.¹⁰ Soluble fibrin monomer complexes and D-dimer levels were measured with immunoturbidimetric assays (STA-Liatest FM; Diagnostika Stago, Asnieres, France; and Tinaquant D-Dimer; Roche Diagnostics GmbH, Mannheim, Germany). Levels of the cardiac biomarkers mass of the Mb fraction of creatine kinase (CK-Mbm) and troponin T (TnT) were determined with electrochemiluminescence immunoassays (Elecsys Ck-Mb-STAT and Elecsys TroponinTSTAT, Roche Diagnostics GmbH). APC, protein C, F1+2, and D-dimer levels were measured from all patients at all time points (see above); soluble fibrin monomer complexes were measured from patients 1 to 20 at time points A to F; and CK-Mbm and TnT levels were measured from all patients at time point G (18 hours postoperatively).

Statistical Analysis

The data were analyzed with the SPSS for Windows 11.5.1 software (SPSS, Inc, Chicago, Ill) and the NCSS 2000 software (NCSS, Kaysville, Utah). For clarity, all data are presented as the mean and standard deviation (SD) or as the mean and standard error of the mean. Analysis of normality of the distribution of continuous variables was performed with the Kolmogorov-Smirnov test. For univariate analysis of the association between variables, the Pearson and Spearman rank correlation coefficients were calculated, as appropriate. Differences between the repeated measurements were analyzed with repeated-measures ANOVA, and post-hoc comparisons were made with the Fisher least-significant-difference multiple-comparison test. Variables with skewed distribution were natural logarithmically transformed before these analyses. For multivariable logistic regression analysis, a forward stepwise method was used. Known preoperative clinical risk factors of CABG (EuroSCORE and the presence of diabetes) and a set of intraoperative variables (aortic crossclamp time, number of distal anastomoses, blood loss during the operation, and cumulative heparin dose) were tested with F1+2 levels at various time points as covariates to identify variables associated with protein C activation.

Results

Different Kinetics of APC and Thrombin and Fibrin Formation

APC levels decreased slightly after the initiation of CPB, and during ischemia, there was only a slight increase in the level of APC. Maximal upregulation of APC levels was observed after heparin neutralization (time point E), when the APC level increased more than 3-fold (Figure 1, A). The peak APC level ranged from 178% to 1267% (mean, 546%; SD, 84%).

Thrombin generation (F1+2) increased steadily during ischemia, and reperfusion further increased the rate of thrombin generation (Figure 1, A). Soluble fibrin monomer complexes were measured from patients 1 to 20 to investigate whether the increase in thrombin generation was associated with synchronous fibrin formation (Figure 1, B). Fibrin monomer complex levels increased in parallel with F1+2 levels, and the rate of fibrin formation exceeded the rate of thrombin generation. Postoperatively (time point F), the ratio of the level of fibrin monomer complexes to F1+2 was more than 2.5-fold compared with the preoperative ratio (Figure 1, C). Protein C activation was clearly delayed in relation to both F1+2 and fibrin formation (Figure 1, A-C). APC/protein C and APC/F1+2 ratios were calculated to further characterize the activation status of protein C. Because of little variation in protein C levels (mean, 72%-95%), changes in APC/protein C ratios were similar to changes in absolute APC levels. Overall, throughout the study, the APC/F1+2 ratio was less than preoperatively (repeated-measures ANOVA: $P < .001$), indicating a dominance of thrombin generation over protein C activation (Figure 1, C).

Three phases of the balance between APC and F1+2 levels could be identified (Figure 1, C). First, after the initiation of CPB and during ischemia (time points A-C), APC/F1+2 ratio decreased by more than 60%, apparently because of increasing thrombin generation without a response by APC. There was therefore a marked delay in the increase in APC levels in relation to F1+2 levels. During early reperfusion (time point D), the APC level already clearly increased, yet the APC/F1+2 ratio further decreased because of a burst in thrombin generation.

Second, after heparin neutralization (time point E), both F1+2 and APC levels peaked, with the APC response being more pronounced, and there was a shift in the APC/F1+2 balance. Thereafter, APC levels decreased more rapidly than F1+2 levels.

Finally, on the first postoperative day (time point G), both APC and F1+2 levels were low, and from postoperative day 1 to 5 (time points G-H), APC levels decreased, whereas F1+2 levels increased, again causing the APC/F1+2 balance to be shifted back toward thrombin dominance.

Determinants of Protein C Activation

In univariate analyses APC levels correlated with F1+2 levels (Table 1) and D-dimer levels (correlation coefficients from $r = 0.33$ to $r = 0.49$, $P < .001$) during CPB and reperfusion. The strength of this correlation increased during CPB and myocardial ischemia and peaked during early reperfusion (time point D). Peak APC and peak APC/protein C levels (time point E) correlated weakly with aortic crossclamp time ($r = 0.31$, $P = .001$ and $r = 0.32$, $P = .016$, respectively) and CPB time ($r = 0.35$, $P < .001$ and $r = 0.36$, $P < .001$, respectively).

Multiple patient- and operation-related clinical variables and F1+2 levels (see “Statistical analysis”) were tested in multivariable logistic regression analysis for associations with protein C activation. F1+2 levels were associated with APC levels at all time points, as expected (data not shown).

APC and Hemodynamic Recovery

APC levels immediately before the release of the aortic clamp had weak positive correlations with CI values after CPB (time point E) and on arrival in the ICU ($r = 0.21$, $P = .037$ and $r = 0.22$, $P = .03$, respectively) and negative correlations with SVRI values measured during the same time points ($r = -0.20$, $P = .04$ and $r = -0.26$, $P = .012$, respectively). APC levels during early reperfusion (time point D) had a weak negative correlation with postoperative SVRI values ($r = -0.25$, $P = .013$). Because the kinetics of protein C activation and thrombin formation differed from each other, we separately analyzed whether the highest or lowest deciles or quintiles of either absolute levels of APC, APC/protein C ratio, or, alternatively, APC/F1+2 balance were associated with postoperative hemodynamic measurements.

High preoperative APC levels were associated with an unfavorable postoperative hemodynamic profile. Patients with preoperative APC/protein C ratios (time point A) in the highest decile had lower CI values postoperatively than others (repeated-measures ANOVA: $P = .026$, Figure 2). Conversely, patients with preoperative APC levels in the lowest decile or quintile had lower PVRI values postoperatively (time points F and G; mean PVRI, 345 vs 225 $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5} \cdot \text{m}^{-2}$ and 246 vs 173 $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5} \cdot \text{m}^{-2}$, respectively; $P = .005$ and $P = .02$, respectively).

At the end of cardiac ischemia, immediately before the release of the aortic clamp (time point C) and during early reperfusion 15 minutes after the release of the aortic clamp (time point D), protein C activation was associated with an opposite and favorable hemodynamic response. Patients with APC levels in the highest quintile before the release of the aortic clamp had higher CI values postoperatively than others ($P = .03$, Figure 3). Those with APC/protein C ratios in the highest quintile during early reperfusion had lower SVRI values postoperatively (mean SVRI, 2137 vs 2429 $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5} \cdot \text{m}^{-2}$; $P = .045$). Consistently, those

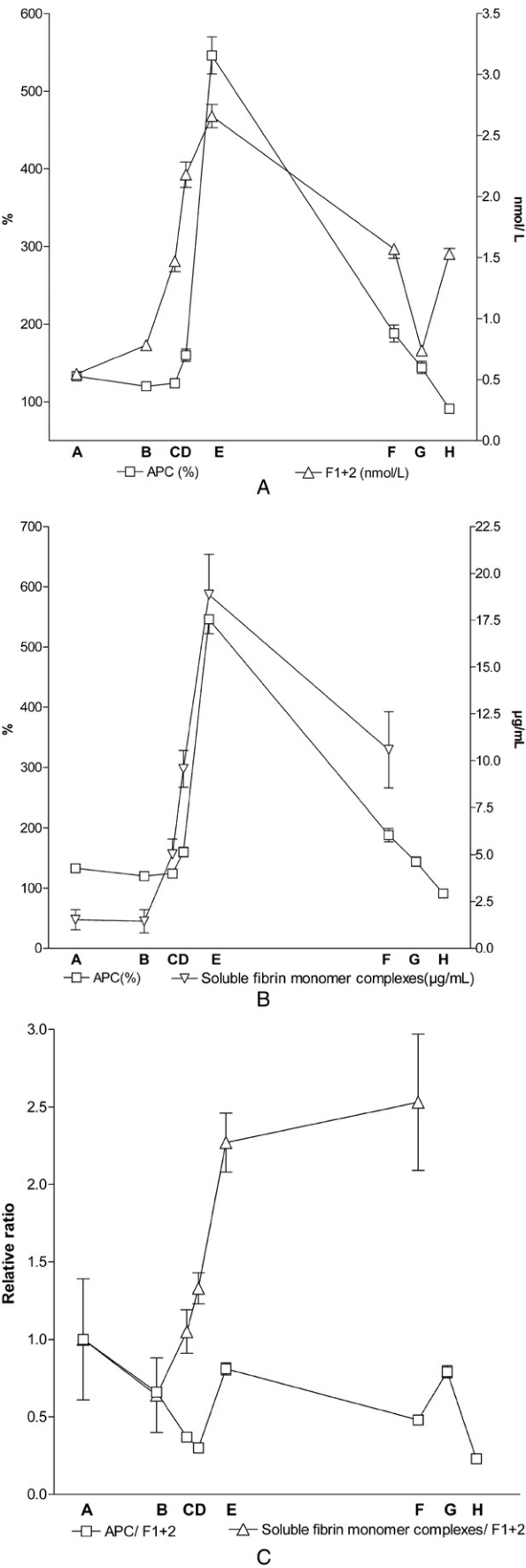


TABLE 1. Correlation between prothrombin fragment F1+2 and activated protein C levels

		Activated protein C							
		A	B	C	D	E	F	G	H
F 1+2	A	0.08							
	B		0.26*						
	C			0.42*					
	D				0.55*				
	E					0.45*			
	F						0.35*		
	G							0.27*	
	H								0.19

Correlation between prothrombin fragment F1+2 and activated protein C at various time points: preoperatively (A), at 15 minutes of CPB (B), immediately before the release of the aortic clamp (C), 15 minutes after the release of the aortic clamp (D), 30 minutes (E) and 6 hours (F) after protamine administration, and on the first (G) and fifth (H) postoperative days. Spearman correlation coefficients are shown. * $P < .01$.

with APC/F1+2 ratios in the lowest decile or quintile before the release of the aortic clamp ($P = .01$ and $P = .007$, respectively) and during early reperfusion ($P = .001$ and $P = .04$, respectively) had higher postoperative PVRI values on arrival in the ICU (data not shown).

After heparin neutralization with protamine (time point E), APC levels were associated again with an inferior hemodynamic profile. Those with APC levels in the highest decile after heparin neutralization had lower SVI values postoperatively ($P = .026$, Figure 4). Conversely, patients who had APC levels in the lowest decile measured during the same time point (time point E) had a trend toward higher postoperative SVI and CI values, which did not reach statistical significance. Those with APC/protein C ratios in the highest decile or quintile after heparin neutralization had higher postoperative PVRI values than others (mean PVRI of highest quintile, 472 vs 343 $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5} \cdot \text{m}^{-2}$; $P = .04$).

Figure 1. Changes in levels of activated protein C (APC; panels A and B), prothrombin fragment F1+2 (panel A),¹⁰ soluble fibrin monomer complexes (panel B), and ratios of APC to F1+2 (APC/F1+2; panel C) and soluble fibrin monomer complexes to F1+2 (panel C). The mean preoperative ratios were arbitrarily adjusted to 1.0 to better illustrate their differential kinetics. Time points measured are preoperatively (A), at 15 minutes of CPB (B), immediately before the release of the aortic clamp (C), 15 minutes after the release of the aortic clamp (D), 30 minutes (E) and 6 hours (F) after protamine administration, and on the first (G) and fifth (H) postoperative days. The time scale reflects actual time between time points A through F. Time points G and H are not in scale. Values are presented as means \pm standard error of the mean.

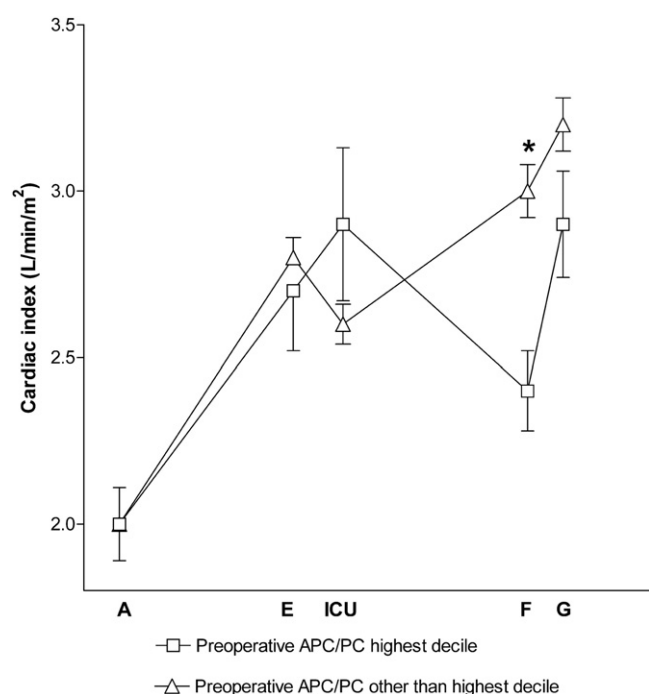


Figure 2. Cardiac index of patients with preoperative activated protein C to protein C ratio (APC/PC) in the highest decile versus others. Time points measured are preoperatively (A), after termination of cardiopulmonary bypass (E), postoperatively on arrival in the intensive care unit (ICU), 6 hours after protamine administration (F), and on the first postoperative day (G). The time scale reflects actual time between time points A through F. Time point G is not in scale. *Repeated-measures analysis of variance: $P < .05$ between groups. Values are presented as means \pm standard error of the mean.

Patients who required norepinephrine and epinephrine on the morning of the first postoperative day had higher peak APC levels (time point E) than others (repeated-measures ANOVA: $P = .007$ and $P < .001$, respectively). Otherwise, use of vasoactive medications was not associated with APC levels.

APC and Myocardial Damage

The mean postoperative CK-Mbm level was $42 \mu\text{g/L}$ (SD, $42 \mu\text{g/L}$), and the mean TnT level was $0.75 \mu\text{g/L}$ (SD, $0.69 \mu\text{g/L}$). APC or protein C levels did not correlate with postoperative cardiac biomarker levels (CK-Mbm or TnT, data not shown). The patients were stratified into groups according to the extent of cardiac biomarker release. Patients with evidence of postoperative myocardial damage defined as either a CK-Mbm level of $100 \mu\text{g/L}$ or greater and a CK-Mbm level of $69 \mu\text{g/L}$ or greater ($>90\text{th}$ and $>80\text{th}$ percentile, respectively) or a TnT level of $1.61 \mu\text{g/L}$ or greater and a TnT level of $1.24 \mu\text{g/L}$ or greater ($>90\text{th}$

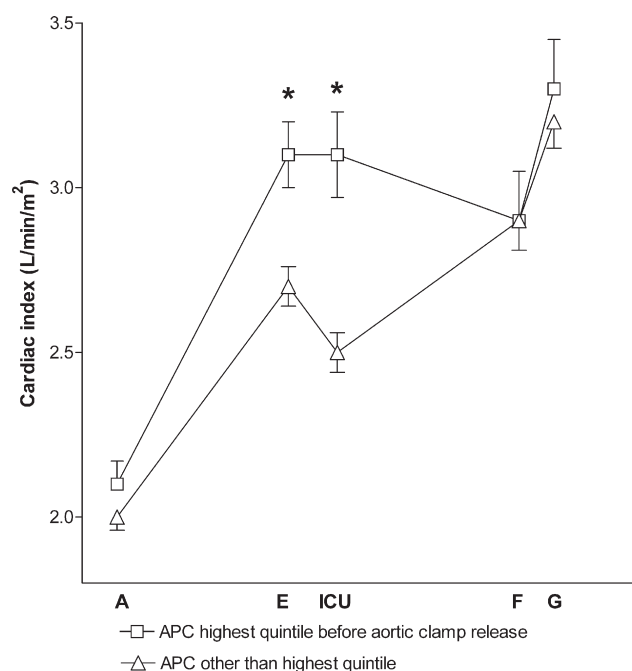


Figure 3. Cardiac index of patients with activated protein C (APC) levels in the highest quintile before the release of the aortic clamp (time point C) versus others. Time points measured are preoperatively (A), after termination of cardiopulmonary bypass (E), postoperatively on arrival in the intensive care unit (ICU), 6 hours after protamine administration (F), and on the first postoperative day (G). The time scale reflects actual time between time points A through F. Time point G is not in scale. *Repeated-measures analysis of variance: $P < .05$ between groups. Values are presented as means \pm standard error of the mean.

and $>80\text{th}$ percentile, respectively) did not have APC or protein C levels different from those of other patients (repeated-measures ANOVA: data not shown). Also, patients with new Q-waves postoperatively ($n = 7$), who were all in the highest quintile of CK-Mbm and TnT levels, did not have APC levels different from those of other patients (repeated-measures ANOVA: data not shown).

Discussion

During CABG, the activation of protein C was associated closely with thrombin generation, and the correlation between APC and F1+2 levels was strongest during early reperfusion. However, thrombin and fibrin formation both preceded and dominated over APC. When an early response of APC to thrombin generation was observed (ie, at the end of ischemia and during early reperfusion), the postoperative hemodynamic profile was favorable. Conversely, high preoperative APC levels and high peak APC levels, which coincided with peak thrombin generation, were associated with unfavorable postoperative hemodynamic performance.

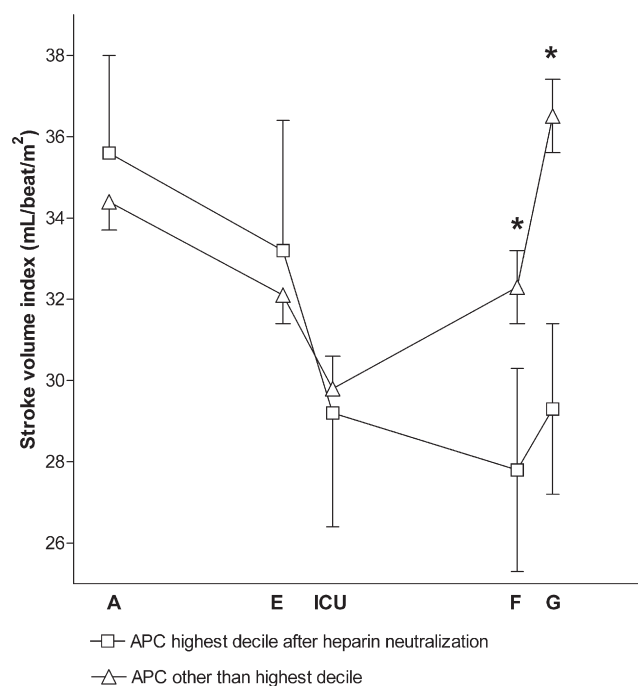


Figure 4. Stroke volume index of patients with activated protein C (APC) levels in the highest decile after heparin neutralization (time point E) versus others. Time points measured are preoperatively (A), after termination of cardiopulmonary bypass (E), postoperatively on arrival in the intensive care unit (ICU), 6 hours after protamine administration (F), and on the first postoperative day (G). The time scale reflects actual time between time points A through F. Time point G is not in scale. *Repeated-measures analysis of variance: $P < .05$ between groups. Values are presented as means \pm standard error of the mean.

The peak APC level observed in this study probably reflects close to the maximal physiologic APC level because it is well comparable with maximal APC levels observed in liver and renal transplantation, which represent clinical settings with strong reperfusion-induced activation of protein C.^{15,16} Also, the maximal APC levels observed in patients with sepsis⁸ or severe pancreatitis,⁹ 2 conditions with systemic inflammation and coagulopathy, were similar or less than the current maximal APC levels. Despite the dynamic association of APC levels with postoperative hemodynamic recovery, there was no association between protein C activation and evidence of postoperative myocardial damage.

Experimental evidence suggests that APC levels might have beneficial effects on ischemia-reperfusion-induced tissue damage. APC has reduced ischemia-reperfusion-induced organ injury in the brain,^{17,18} spinal cord,¹⁹ kidney,²⁰ intestine,²¹ and myocardium.²² It could be expected that in a clinical situation after myocardial ischemia and reperfusion and myocardial stunning, activation of protein

C would be associated with enhanced myocardial recovery. In a previous smaller study protein C activation during early reperfusion after CABG was associated with improved hemodynamic recovery after CABG.¹¹ This finding is now confirmed. However, the unfavorable associations between preoperative and peak APC levels and postoperative hemodynamics in the present study were unexpected. Even though the explanation remains beyond the scope of the present study, high APC levels before surgical intervention might indicate a secondary response to an underlying thrombin challenge produced by a stressed and more pathologic endothelium throughout the vasculature. If so, the distorted thrombin-APC balance could logically be associated with unfavorable hemodynamic performance after surgical intervention. We have previously shown that thrombin generation during reperfusion was associated with myocardial damage and that thrombin generation after heparin neutralization was associated with increased postoperative pulmonary resistance.¹⁰ Therefore the observed association between peak protein C activation after heparin neutralization and an unfavorable hemodynamic profile might be logical if we assume that the response by APC to the thrombin challenge observed was maximal but insufficient to reverse the possible negative effects of thrombin. Also, other mediators could be involved. Thrombin activates thrombin-activatable fibrinolysis inhibitor, the role of which is unknown during CPB. It is possible that during a burst and propagation of thrombin generation, activation of thrombin-activatable fibrinolysis inhibitor could lead to reduced fibrinolysis in the microcirculation, which, in turn, could be associated with hemodynamic control independently of protein C activation.

We did not find an association between APC levels and postoperative myocardial damage. A previous clinical study that examined the association of APC levels with the responsiveness to thrombolytic therapy for acute myocardial infarction (AMI) found an association with increased APC level and unsuccessful thrombolysis.²³ Patients with ST-segment elevation AMI had higher APC levels than control subjects, and patients with failed infarct-related coronary artery recanalization had higher APC levels than patients with successful thrombolysis after AMI. However, rather than ST-segment elevation AMI, our patients had postoperative cardiac biomarker increase, the mechanism of which is known to differ from that of cardiac biomarker increase in AMI not related to CABG.²⁴

The present study has some limitations. It was designed to be large enough to detect possible associations between APC levels and postoperatively measured continuous variables (ie, hemodynamic measurements and the levels of cardiac biomarkers) and could have been underpowered to detect an association between APC levels and postoperative AMI. Furthermore, the study design is descriptive and does

not allow direct conclusions to be made about the possible mechanisms of the associations detected.

We conclude that this is the first study of its size to demonstrate an association between preoperative and intra-operative APC levels and APC/thrombin balance and post-operative sequelae after CABG. The study is novel in demonstrating that protein C activation is delayed in relation to thrombin generation and that protein C activation is associated dynamically with hemodynamic recovery after CABG. Because thrombin generation during reperfusion after CABG has been previously implicated to be associated with myocardial damage and compromised hemodynamic recovery,¹⁰ the finding that APC levels are associated with hemodynamic recovery after CABG is logical. Overall, the data add to the evidence that suggests that hypercoagulation after CABG, especially during reperfusion, might be a clinically important phenomenon.

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